In response to the Examiner's objections, Applicants have amended claims 10, 21 and 17 to correct spelling and typographical errors.

2. The Claims as Amended Are Definite

The Examiner has rejected claims 2, 15 and 21 under 35 U.S.C.§112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Examiner alleges that the wording "the cytotoxic agent is irradiation" in claims 2 and 15 is awkward.[1] In addition, the Examiner maintains that in claim 21 their is insufficient antecedent basis for the limitation "wherein the modulator is salicylihalamide A."

Applicants have amended claim 2 to encompass "a method of promoting cell death following exposure to irradiation comprising contacting said cell with a modulator of vacuolar proton ATPase activity." Applicants have amended claim 15 to encompass " a method of promoting cell death following exposure to irradiation comprising contacting said cell with an agent capable of inhibiting acidic vesicular function or acidification." In addition, claim 21 has been amended to replace the term "modulator" with "agent", thereby correcting the antecedent basis.

[1] Applicants assume that the Examiner meant to refer to claim 3, rather than claim 2, since claim 2 does not refer to irradiation.

3. The Rejections Under 35 U.S.C.§103 Should Be Withdrawn

Claims 1, 4-8, 11-14 and 16-19 are rejected under 35 U.S.C. §103 (a) as being unpatentable over Altan et al., (1998, J. Exp. Med. 187:1583-1598:"Altan") in view of Bechimol et al., (1998, Biochem. J. 332:695-702:"Bechimol").

Claims 2 and 15 are rejected under 35 U.S.C. §103 (a) as being unpatentable over Teicher et al., (1996, Radiation Oncology Investigations 4:221-230;"Teicher") in view of Furuya et al., Cancer Res. 54:6167-6175;"Furuya").

Claims 9-10 and 20-21 are rejected under 35 U.S.C. §103 (a) as being unpatentable over Altan in view of Boyd et al., (2001, Journal of Pharmacology and Experimental Therapeutics 297:114-120;"Boyd").

The Court of Appeals for the Federal Circuit (CAFC) summarized the legal standard with regard to the showing necessary to support a proper rejection under Section 103 in *In re Rijckaert*, 28 USPQ2d 1955, (1993) as follows:

> "In rejecting claims under 35 U.S.C.§103 , the Examiner bears the initial burden of presenting a prima facie case of obviousness....A prima facie case of obviousness is established when the prior art itself would appear to have suggested the claimed subject matter to a person of ordinary skill in the art. If the Examiner fails to establish a prima facie case, the rejection is improper and will be overturned."

The prior art relied upon by an Examiner to establish a prima facie case must not only suggest that the claimed method be performed, but the prior art must also provide one of

ordinary skill in the art with a reasonable expectation that the claimed subject matter can be successfully used to effect a practical purpose. *In re Vaeck* ,20 USPQ2d 1438 (Fed. Cir. 1988)

With regard to the Examiner's rejection of claims 1, 4-8, 11-14 and 16-19 under 35 U.S.C.§ 103, the relevant inquiry is whether Altan in combination with Bechimol renders obvious the presently claimed methods of promoting cell death.

The Examiner alleges that Altan teaches a method of promoting cell death following exposure to adriamycin, a cytotoxic agent, comprising contacting cells with modulators of vacuolar proton ATPase activity. The Examiner maintains that Bechimol discloses a method of inhibiting vacuolar-type H+-ATPase expression and inhibition of vacuolar-type H+-ATPase subunit expression by contacting a cell with bafilomycin A1.

Applicants assert that Altan in combination with Bechimol fails to render obvious the subject matter encompassed by claims 1, 4-8, 11-14, and 16-19. First, Altan relates to defective acidification in *drug-resistant cells*. A review of Altan indicates that the reference merely discloses that the pH inside the acidic organelles of *drug resistant cells* is lower than that observed in the acidic organelles of drug-sensitive cells. Thus, in *drug-resistant cells* weakly *basic* chemotherapeutic agents such as doxorubicin are sequestered in the cell's acidic compartments thereby limiting the amount of drug that will reach the nucleus. Indeed, as stated on page 1586, second column of Altun, "since adriamycin is a weak base, it is predicted to accumulate inside acidic compartments." Given these results, it is not surprising that treatment of drug resistant cells with disrupters of organelle acidification, such as bafilomycin, lead to a decrease in the sequestering of weakly basic drugs thereby increasing the sensitivity of such cells to chemotherapeutic agents.

Significantly, Altan also demonstrates that in *drug sensitive* cells, doxorubicin is diffuse throughout the cytoplasm and nucleus, presumably because the pH of the acidic compartments is not as low as that observed in *drug resistant* cells. Given these results, one of skill in the art would not be lead to reasonably believe that treatment of such drug sensitive cells with disrupters of organelle acidification, such as bafilomycin, would have any effect on chemotherapeutic or radiation sensitivity. Therefore, Altan fails to provide a teaching that would suggest the use of V-H+-ATPase inhibitors for promoting cell death following exposure to cytotoxic agents.

Furthermore, Applicants assert that Bechimol fails to provide the suggestion that is missing from the Altan reference, *i.e.*, that inhibitors of V-H+-ATPases increase the sensitivity of cancer cells to cytotoxic agents. Additionally, it is noteworthy that Bechimol merely discloses a method of inhibiting vacuolar-type H+-ATPase *functional* expression but fails to demonstrate inhibition of protein expression.

The Examiner has rejected claims 9-10 and 20-21 under 35 U.S.C. §103 (a) as being unpatentable over Altan in view of Boyd et al., (2001, Journal of Pharmacology and Experimental Therapeutics 297:114-120;"Boyd"). The Examiner alleges that Altan teaches a method of promoting cell death following exposure to adriamycin, a cytotoxic agent, comprising contacting cells with modulators of vacuolar proton ATPase activity. Further, although Altan does not teach the use of vacuolar ATPase inhibitors such as benzolactone enamides to promote cell death, Boyd teaches benzolactone enamides as inhibitors of tumor cell growth due to inhibition of vacuolar type (H+) ATPases.

As indicated above, the Altan reference fails to teach or suggest that inhibitors of V-H+-ATPases can be used to increase the sensitivity of cancer cells to cytotoxic agents.

Applicants assert that the Boyd reference also fails to provide the teaching or suggestion that is absent from Altan. Boyd merely discloses that antitumor benzolactone enamides selectively inhibit mammalian vacuolar type (H+) ATPases.

Claims 2 and 15 are rejected under 35 U.S.C. §103 (a) as being unpatentable over Teicher et al., (1996, Radiation Oncology Investigations 4:221-230;"Teicher") in view of Furuya et al., Cancer Res. 54:6167-6175;"Furuya"). According to the Examiner, Furaya teach a method for promoting cell death comprising contacting said cell with the art known ATPase inhibitor, thapsigargin. Further, Teicher teaches a method of promoting cell death comprising irradiating a cell and contacting said cell with thapsigargin.

Applicants assert that Teicher in combination with Furaya fail to render obvious the subject matter encompassed by claims 2 and 15, *i.e*, methods for promoting cancer cell death following exposure to a modulator of vacuolar proton ATPase activity (claim 2) or an agent capable of inhibiting acidic vesicular function (claim 15). Both Teicher and Furaya relate to methods comprising exposure of cells to thapsigarden. However, thapsigarden is not a vacuolar protein ATPAse, or an agent capable of inhibiting acidic vesicular function, but rather, an endoplasmic reticulum or sarcoplasmic reticulum intracellular Ca2+ pumping ATPase. Furthermore, Teicher teaches that administration of thapsigargin provided little or no tumor growth delay compared with radiation therapy alone (see abstract and p.224, second column of Teicher). In view of the differences between the two types of ATPase pumps, the invention encompassed by claims 2 and 15 cannot be rendered obvious in view of Teicher or Furaya, either alone, or in combination.

Applicants assert that the claimed invention is not obvious in view of any of the references cited by the Examiner. Therefore, Applicants respectfully request withdrawal of the rejections under 35 U.S.C.§103.

CONCLUSION

Entry of the foregoing amendments and remarks into the file of the above-identified application is respectfully requested. Applicants believe that the invention described and defined by the amended claims is patentable over the rejections of the Examiner. Withdrawal of all rejections and reconsideration of the amended claims is requested. An early allowance is earnestly sought.

Respectfully submitted,

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IN THE CLAIMS

3. (amended) [The method of claim 1 wherein the cytotoxic agent is irradiation] A method of promoting cell death following exposure to irradiation comprising contacting said cell with a modulator of vacuolar proton ATPase activity.

10. (amended) The method of claim 9 wherein the modulator is [salicilyhalamide] salicylihalamide A.

15. (amended) [The method of claim 1 wherein the cytotoxic agent is irradiation] A method of promoting cell death following exposure to irradiation comprising contacting said cell with an agent capable of inhibiting acidic vesicular function or acidification.

17. (amended) The method of claim 13 wherein the agent is [an] a macrolide.

21. (amended) The method of claim 20 wherein the [modulator] agent is [salicilyhalamide] salicylihalamide A.